FACULTY OF MEDICINE AND HEALTH SCIENCES

Novel peptide tools to inhibit the IP_3 receptor/ connexin-43 hemichannel calcium signaling axis

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Summary

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are ubiquitous, large, tetrameric intracellular Ca^{2+} release channels primarily located at the membrane of the endoplasmic reticulum (ER) of biological cells. They are a basic constituent of the IP_3-Ca^{2+} signaling pathway that serves as a fundamental mechanism for transmitting signals and regulating a wide range of cellular processes and physiological responses. Mammalian cells express three distinct IP_3R subtypes (IP_3R1, IP_3R2 and IP_3R3), which possess ~70% homology despite originating from different genes. Currently, most therapeutic IP_3R antagonists have shortcomings in terms of selectivity and side effects. The first challenge in this PhD thesis work was to obtain molecular tools to interfere with IP_3R function without affecting connexin-based channels.

Thus, in the ***first*** part of this PhD thesis, inspired by recent work that revealed a regulatory mechanism in type-3 IP_3Rs (IP_3R3) whereby a loop extending from ARM2 between the α_{ARM2} -1 and α_{ARM2} -2 domain occupies the IP_3-binding site and competitively inhibits IP_3 binding, we identified a novel peptide tool called IP3RPEP6 composed of a sequence located at the end of the ARM2 structure of IP_3R2 that interacts with the IP_3-binding site and competitively inhibits IP_3-binding and subsequent activation of Ca^{2+} channel opening. Our results indicate that IP3RPEP6 inhibits the activation of IP_3R2 and IP_3R3 with more limited effects on IP_3R1. Importantly, IP3RPEP6 does not affect connexin-43 (Cx43) hemichannels (HCs) nor ryanodine receptor (RyR) activation, which is another intracellular Ca^{2+} release channel. As such, IP3RPEP6 is not compromised by side effects associated with other IP_3R inhibitors and enables the accurate observation and assessment of IP_3R-specific responses.

The IP_3-Ca^{2+} signaling axis has many cross-points with connexins. Activation of IP_3R signaling modulates gap junctional coupling but also affects hemichannels, i.e. the precursor channels of gap junctions, which are more strongly activated to open in response to IP_3-triggered $[Ca^{2+}]_i$ elevation than by Ca^{2+} entry. Given that IP_3-Ca^{2+} signaling efficiently activates HC opening, we ***next*** investigated whether HC opening could contribute to the cellular Ca^{2+} responses induced by IP_3 elevation. We found that IP_3-induced Ca^{2+} changes also contained a component resulting from Cx43 HC opening, which altered the EC_{50} and Hill slopes of carbachol-induced IP_3-dependent Ca^{2+} responses. Therefore, Cx43 HCs should be blocked to reliably assess the properties of IP_3-triggered Ca^{2+} responses and the effect of IP_3R inhibitors on these responses, to ensure an unbiased assessment of the pharmacological properties derived from such experiments.

Recent research in host lab revealed that activation of type-2 RyRs (RyR2) also activates downstream opening of Cx43 HCs. Such activation depended on molecular interactions between RyR2 and Cx43 in ventricular cardiomyocytes. Interestingly, IP_3Rs have also been reported to interact and co-immunoprecipitate with Cx43 in ventricular cardiomyocytes. Therefore, in the ***second*** part of this thesis, we aimed to determine whether there is a direct protein-mediated link between the activation of IP_3R2 and Cx43 HC opening. Our results delineate a direct protein-protein interaction between IP_3R2 and Cx43 in Cx43 overexpressing HEK-293 cells and astrocytes, and further provide a new peptide tool IP_3RHCIp which has a micromolar affinity with the CT tail of Cx43. We subsequently showed that IP_3RHCIp inhibits Cx43 HC opening triggered by IP_3R activation by preventing HC activation.

In conclusion, my work demonstrates the intimate linkage between IP_3-Ca^{2+} signaling and signaling through Cx43 HCs. We developed new peptide tools allowing interference with these two prominent players of Ca^{2+} signaling, charting a new inspirational wave of research focused on determining the contribution of these two players in astrocytic as well as cardiomyocyte signaling.

Examination board

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Selected publications

(*shared authorship)

Tao S, Hulpiau P, de Ridder I, Witschas K, Bultynck G, Leybaert L. An IP3 receptor-based peptide interfering with IP3/calcium induced opening of Cx43 hemichannels. *In preparation for submission*

Tao S, Hulpiau P, Wagner L.E, Witschas K, Yule D.I, Bultynck G, Leybaert L. IP3RPEP6, a novel peptide inhibitor of IP3 receptor channels that does not affect connexin-43 hemichannels. *Acta Physiologica*. 2024; 10.1111/apha.14086

Lissoni A*, **Tao S***, Allewaert R, Witschas K, Leybaert L. Cx43 Hemichannel and Panx1 Channel Modulation by Gap19 and 10Panx1 Peptides. *International Journal of Molecular Sciences*. 2023; 24(14):11612

Patent

Patent number EP 23169580.0 at European Patent Office (EPO), 2023-2024: Inositol 1,4,5-trisphosphate receptor inhibitor peptides and uses thereof

Short Curriculum Vitae

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